Literature Review

Prenatal Diagnosis of Thalassemia

Vlad-Ioan OLTEAN[1,2]

[1]Faculty of Medicine, University Lucian Blaga Sibiu
[2]Hematology Department, Emergency Hospital, Sibiu, Romania

Correspondence to: Oltean Vlad-Ioan e-mail: vlad.oltean9813@gmail.com

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Abstract

Thalassemia is a group of inherited blood disorders that affect the production of hemoglobin. The main components of hemoglobin consist of heme and globin (α-globin and β-globin). Thalassemia occurs when there is a mutation in the genes responsible for producing these globin chains, leading to an abnormal or insufficient production of one or both types of globin. Depending on the affected globin genes, α- and β-thalassemias can be identified. Depending on the genotype, the clinical presentation of the patient can vary from asymptomatic to severe and also lethal courses. The main and also leading symptom is microcytic hypochromic anemia. Without a treatment, increased erythronpoiesis can induce extramedullary hematopoiesis, hepato-splenomegaly and growth disturbances.

In the context of α-thalassemias distinctions are made among the minor and minima form (expressed by few symptoms), HbH disease (manifesting mild to moderate symptoms) and Hb Barts hydrops fetalis syndrome (showing severe symptoms and also often resulting in perinatal death). The choice of treatment depends on the specific clinical presentation and closely mirrors the approach taken for β-thalassemias.

In the case of β-thalassemias, a differentiation is made between the minor form (also with few symptoms) and the homozygous major form. Without a treatment the latter leads to severe outcomes in childhood. The symptomatic therapy involves transfusions and efforts are made to mitigate the life-limiting complications of secondary iron overload by administering iron chelators (e.g. Deferoxamine). Causal therapy is possible through a stem cell transplant, or experimentally through gene therapy.

Introduction

Various mutations affecting hemoglobin have been discovered so far. Thalassemias can lead to severe anemia from an early age, and without regular blood transfusions, they can result in death within the first year of life. Prenatal detection of thalassemia is a crucial component of preventive medicine, typically relying on invasive diagnostic tests during the initial two months of pregnancy[1]. However, these diagnostic techniques carry a small yet noteworthy risk of fetal loss, around 1%. Molecular diagnostic approaches for genotyping thalassemias have been developed, employing PCR methods and advanced high-throughput technologies. Another alternative method involves noninvasive testing, utilizing cell-free fetal DNA (cffDNA) obtained from a maternal blood sample, effectively eliminating the risk of miscarriage[2].

Epidemiology

Thalassemia has a global incidence of approximately 4.4 cases per 10,000 live births. It is noteworthy that both males and females inherit the responsible gene mutations equally, as thalassemia follows an autosomal pattern of inheritance, showing no gender preference.

Roughly 5% of the global population exhibits variations in either the alpha or beta components of the hemoglobin molecule. However, it's important to note that not all of these variations lead to symptoms, and some individuals are classified as silent carriers. In reality, only 1.7% of the worldwide population displays symptoms attributable to gene mutations, a condition referred to as thalassemia trait.

Nonetheless, certain ethnic groups have a higher likelihood of being affected, with prevalence rates of thalassemia symptoms ranging from 5% to 30% within these populations.

Alpha-thalassemia is notably prevalent in specific ethnic groups with Southeast Asian roots. Additionally, there's a substantial presence of carriers in Sub-Saharan Africa and the Western Pacific regions. The distribution of these various population groups varies based on the geographical regions around the world, and it can be outlined as follows:

- **America**: 0-5% of the population has a thalassemia trait, up to 40% of this population possibly being genetic carriers.
- **Eastern Mediterranean**: 0-2% of the population has a thalassemia trait, with up to 60% of this population potentially being genetic carriers.
- **Europe**: 1-2% of the population has a thalassemia trait, with up to 12% of this population being genetic carriers.
- **Southeast Asia**: 1-30% of the population has a thalassemia trait, with up to 40% of this population potentially being genetic carriers.
- **Sub-Saharan Africa:** 0% of the population has a thalassemia trait and up to 50% of this population potentially being genetic carriers.
- **Western Pacific**: 0% of the population has a thalassemia trait, with up to 60% of this population potentially being genetic carriers.

Among populations with Mediterranean, African, and South Asian heritage, beta-thalassemia stands as the most prevalent form of thalassemia. The distribution of this condition among various population groups around the world can be outlined as follows based on geographical regions:

- **America**: 0-3% of the population is affected by a gene mutation
- **Eastern Mediterranean**: 2-18% of the population is affected by a gene mutation
- **Europe**: 0-19% of the population is affected by a gene mutation
- **Southeast Asia**: 0-11% of the population is affected by a gene mutation
- **Sub-Saharan Africa**: 0-12% of the population is affected by a gene mutation
- **Western Pacific**: 0-13% of the population is affected by a gene mutation[3].

Hemoglobinopathies

Hemoglobin (Hb) serves as the molecule responsible for transporting oxygen in red blood cells. In each adult Hb molecule, there are 4 subunits, comprising 2 α-globin and 2 β- (or β-like) globin chains. The α-globin gene cluster is located close to the telomere on the short arm of chromosome 16. On the other hand, the human β-globin spans a region of approximately 70 kb situated on the short arm of chromosome 11 and encompasses five functional genes. Hb A is the dominant form of Hb molecule found in adult humans.

While the α-globin gene cluster experiences a single developmental "switch," the β-gene cluster goes through 2 such transitions. During the embryonic stage, transcription begins with the ε gene but switches to the transcription of the two γ genes after the sixth week of gestation, primarily occurring in the fetal liver and continuing into the prenatal period. Subsequently, this transcription transitions to the δ (minor adult) and β (major adult) genes. Approximately six months after birth, the presence of hemoglobin F (HbF) constitutes less than 5% of the total hemoglobin and gradually decreases, eventually reaching less than 1% in two-year-old individuals.

Inherited hemoglobin disorders constitute extensive groups of autosomal recessive conditions. These disorders arise from over 700 identified defects in globin genes. Autosomal recessive inherited disorders result from either faulty or absent production of one of the globin chains within the Hb tetramer. The specific affected globin chain distinguishes between α-, β-, and δ-thalassemias. The term "thalassemia" originates from the Greek words "thalassa," meaning "sea," and "aemia," indicating "anemia." It was noted that thalassemia is more prevalent in regions where malaria was historically present or endemic.

The underlying pathophysiology of these disorders is rooted in the resultant imbalance in the α:β chain ratio. Both α-thalassemia and β-thalassemia have a notable prevalence in various populations, with β-thalassemia being more widespread and common. β-Thalassemia belongs to a family of inherited hemoglobin disorders characterized by a reduction in the synthesis of β-globin chains. The high frequency of thalassemia can be attributed to the protective advantage it provides against malaria in carriers, similar to the heterozygote advantage

observed in sickle cell hemoglobin carriers. Thalassemias can lead to severe anemia from early in life, and without regular blood transfusions, they can result in death within the first year[2].

Prenatal Diagnosis

The introduction of prenatal diagnosis has provided couples at risk of major hemoglobinopathies with a new avenue and has transformed the approach to screening and counseling for thalassemias. The initial and crucial step in thalassemia prevention involves prenatal diagnosis of these blood disorders. Currently, traditional methods like amniocentesis, chorionic villus sampling (CVS), and cordocentesis are still employed for prenatal thalassemia diagnosis. However, it's important to note that these conventional techniques carry a risk of fetal miscarriage, which is estimated at around 1%.

The American College of Obstetricians and Gynecologists (ACOG) recommends specific steps for managing individuals with certain blood-related conditions:

1. For women exhibiting a low mean corpuscular volume (MCV), ACOG suggests assessing serum ferritin levels. If their levels are normal but they have microcytic anemia, further evaluation through hemoglobin electrophoresis testing is advised.

2. In cases of normal hemoglobin electrophoresis and Asian ancestry, genetic testing for α-thalassemia is recommended. In all these scenarios, it is essential to conduct partner testing to assess the risk of a fetus being affected. Hemoglobin electrophoresis or genetic testing, as appropriate for β- and α-thalassemia, respectively, is utilized for this purpose.

3. Couples identified as carriers of these conditions should undergo genetic counseling to determine the potential risk of having an affected fetus. Additionally, family decision support should be made available to them.

4. The most severe form of α-thalassemia, characterized by the deletion of all four α-genes, results in a condition known as hydrops fetalis. This condition is marked by severe anemia (hemoglobin levels ranging from 3-8g/dL), organ enlargement, and edema. Typically, it leads to fetal demise within the uterus due to heart failure, resulting in preterm labor, stillbirth, and adverse effects on maternal health. Prenatal screening can identify at-risk fetuses, and amniocentesis or chorionic villus sampling can confirm the diagnosis. In suspected cases, Doppler assessments of cerebral vessel flow velocities or direct fetal blood sampling can be utilized to quantify anemia. If intrauterine transfusions are administered promptly, some of these fetuses may survive but would require postnatal transfusion support, classified as α-thalassemia major.

It is important to note that individuals of African ancestry commonly exhibit 2 α-gene deletions; however, these mutations typically occur on different chromosomes (trans configuration). Consequently, the risk of having a fetus with all 4 genes deleted is lower than in individuals with East Asian ancestry, where the 2 gene deletions more frequently occur on the same chromosome (cis configuration).

For women identified as carriers of β-thalassemia trait or HbS trait, partner testing involving hemoglobin fractionation is crucial to assess the fetal risk of β-thalassemia or sickle cell disease.

Before conception, ACOG advises genetic testing and counseling for couples who are at a high risk of thalassemia. For couples who prefer to avoid elective termination, options like in vitro fertilization and preimplantation genetic diagnosis can be considered. In cases where couples were not identified as high risk for thalassemia before pregnancy, the option of DNA testing through chorionic villus sampling or amniocentesis should be presented. Counseling should be offered in all such scenarios. This approach has significantly decreased the incidence of infants born with thalassemia in regions like the Mediterranean, the Middle East, parts of the Indian subcontinent, and Southeast Asia. Ongoing research is exploring innovative methods to reduce the need for invasive procedures like chorionic villus sampling and amniocentesis, including the collection of fetal DNA from fetal cells in maternal blood or plasma.

Ongoing research is focused on non-invasive prenatal detection (NIPD) of α and β thalassemia. The primary challenge in NIPD lies in distinguishing cell-free fetal DNA from maternal DNA. Depending on the parental mutations, 3 primary approaches have been employed to address this challenge: excluding the paternal mutation type, utilizing single-nucleotide polymorphism (SNP) based methods to differentiate the DNA origin, and applying relative mutation dosage (RMD) to identify fetal mutations based on the maternal allele ratio. RMD methods, often utilizing real-time PCR (RT-PCR) to determine the cycle threshold (Ct), which represents the number of PCR cycles required to reach a specific amount of PCR product. In contrast to the traditional Ct approach, this study employed surface-enhanced Raman spectroscopy (SERS) for quantifying thalassemia alleles.

SERS is a scattering spectroscopy technique that detects inelastic vibrations in the secondary structure of molecules, and it possesses the sensitivity to detect even single molecules. This high sensitivity is attributed to plasmonic effects that occur when target molecules adhere to noble metal surfaces. SERS can detect DNA sequences directly or indirectly by measuring Raman-active tags attached to target sequences.

Notably, approximately 3-20% of maternal plasma DNA consists of cffDNA derived from trophoblastic cells, which have shorter sequences than maternal DNA. Consequently, specific amplification and treatment steps are necessary for detecting cffDNA in plasma. Previous approaches have utilized PCR methods for simultaneous detection of three target strands from epizootic pathogens. For mutation detection, methods like mutation-specific PCR and PCR-like techniques such as exponential strand displacement amplification (SDA) and ligase detection reaction (LDR) have been introduced prior to the implementation of SERS. These processes amplify only the mutated sequences and then detect them using SERS. The multiplex PCR method with fluorescence-labeled primers, allows simultaneous amplification of both the SEA mutation and wild-type (WT) alleles. This is particularly useful for comparing the ratios between SEA and WT alleles, and therefore, it was chosen as the pre-treatment method for maternal plasma before SERS measurement[2,4].

Therapy

In the case of minor and minima forms, therapy is typically not necessary. For other forms, regular red blood cell transfusions and possibly the use of iron chelators are indicated based on the clinical situation.

Treatment of α-thalassemias:

- Minima and Minor forms: Usually no therapy is required.
- HbH disease: Intermittent transfusion of red blood cell concentrates may be considered.
- Hb Barts hydrops fetalis syndrome: Usually none, as the decision is often made to terminate the pregnancy or the newborns typically do not survive the perinatal period.

Treatment of β-Thalassemia major:

- Symptomatic treatment options: Symptomatic management of Thalassemia major includes regular red blood cell transfusion therapy combined with chelation therapy to prevent iron overload in the body[1].
- Transfusion of red blood cell concentrates:
  - Procedure: Regular transfusions, typically every 3 weeks, administered alongside combined chelation therapy to avoid secondary iron overload.
  - Initiation of therapy: When repeated hemoglobin (Hb) levels are <8 g/dL or clinically significant symptoms are present.
- Chelation therapy in Thalassemia:
  - Initiation of therapy: Serum ferritin concentrations >1000 µg/L
  - Goal: Prevention and treatment of secondary iron overload.
- Application: Administration of iron chelators as monotherapy or combination therapy, for example, Deferoxamine.

Summary

The identification of individuals with thalassemia syndromes and thalassemia trait has become a crucial health issue. This identification serves the dual purpose of enabling early and comprehensive care while also preventing unnecessary interventions. Early diagnosis is not only essential for preparing at-risk couples but also for identifying fetuses and newborns who may be at risk. Although newborn screening for hemoglobin disorders, initially focused on sickle cell disease, has made significant progress, there is still room for improvement in order to achieve the objective of optimal screening for thalassemia and the establishment of early diagnoses to enhance management strategies[4].

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